

Applicants: Michael Ciallo et al.
Application No.: 09/375,924
Filed: August 17, 1999
For: GENERATION OF MODIFIED MOLECULES
WITH INCREASED SERUM HALF-LIVES
Sheet 1 of 3

AB(IX-2 CIP CPA)

EL939026365US

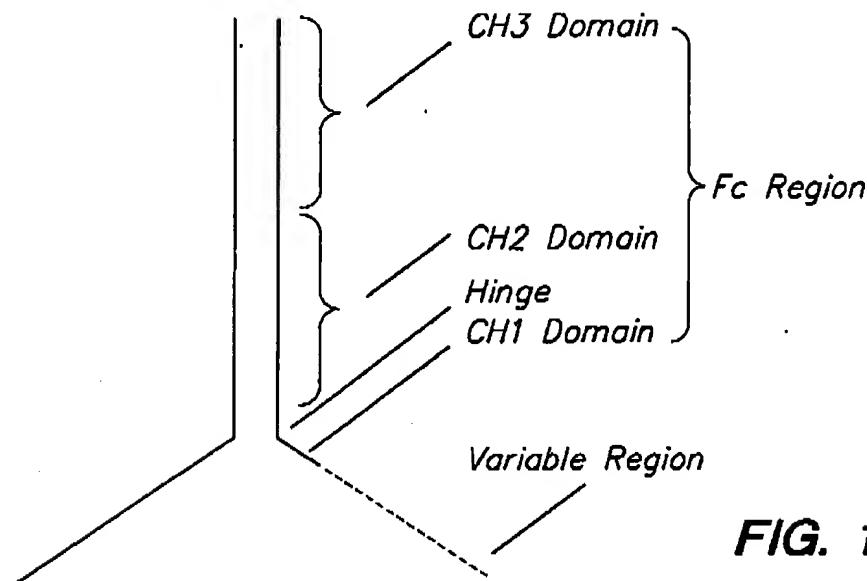


FIG. 1A

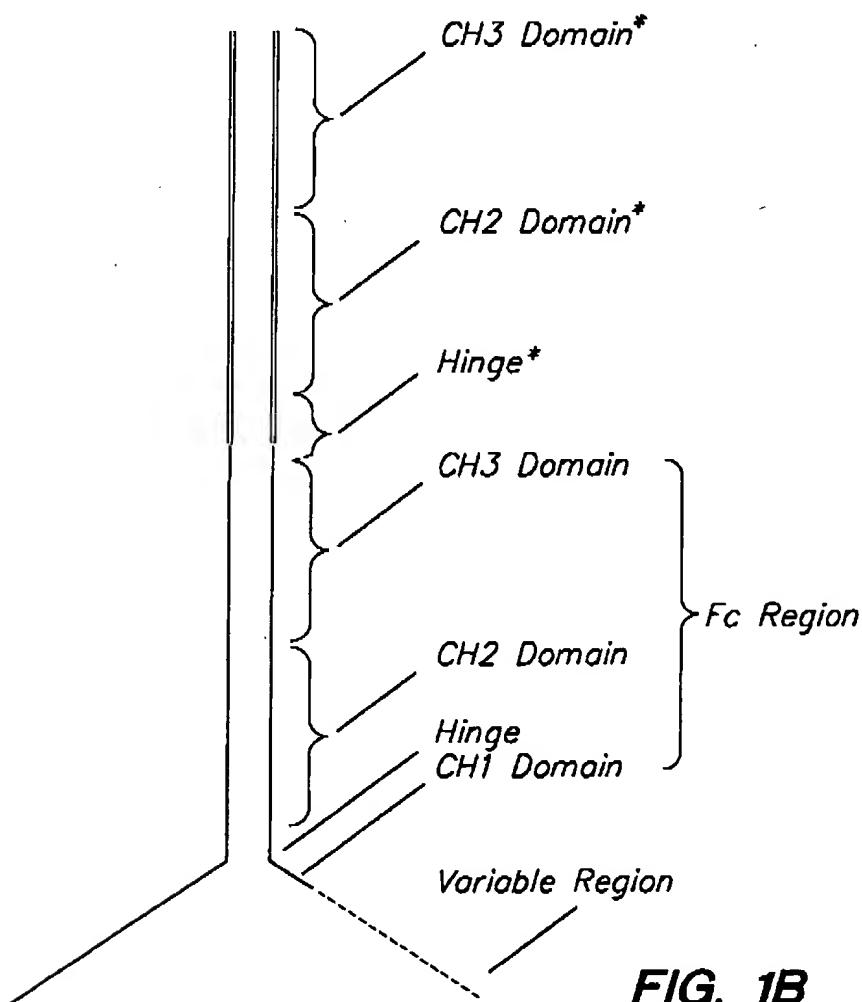


FIG. 1B

Applicants: Michael Gallo et al. ABGX-2 CIP CPA
 Application No.: 09/375,924
 Filed: August 17, 1999
 For: GENERATION OF MODIFIED MOLECULES
 WITH INCREASED SERUM HALF-LIVES
 Sheet 2 of 3

STEP 1: CREATION OF UNIQUE Bsu36I RESTRICTION (CCTT~~AGG~~) SITE IN 3' TERMINUS OF GAMMA-4 CONSTANT REGION:

SEQUENCE ENCODING LAST 4 AMINO ACIDS OF HUMAN GAMMA-4 CONSTANT REGION:

TCT CTG GGT AAA [SEQ ID NO.:5]

MODIFIED SEQUENCE ENCODING THE SAME AMINO ACIDS:

TCC TTA GGG AAG [SEQ ID NO.:6]

STEP 2: PCR PRIMERS UTILIZED FOR ACCOMPLISHING SUCH MODIFICATION:

PRIMER 1:

5'γ4 OLIGO GGG ACC CAC GGG GTG CGA GGG C (Dra III) [SEQ ID NO.:7]

PRIMER 2:

3'γ4 OLIGO CTT CCC TAA GGA CAT GGA GAG GCT CTT CTG TGT GTG (Bsu36I) [SEQ ID NO.:8]

PRIMER 3:

5'γ1 OLIGO GAT TCC TTA GGG AAG GCA GAG CCC AAA TCT AGT GAC (Bsu36I) [SEQ ID NO.:9]
 ser

PRIMER 4:

3'γ1 OLIGO GCC GGA ATT CGG TAC GTG CCA AGC ATC CTC GTG C (EcoR I) [SEQ ID NO.:10]

STEP 3: THREE WAY LIGATION:

- INTRODUCE NEW Bsu36I SITE AT GAMMA 4-HINGE JUNCTION
- ADD HINGE AND GAMMA 1 CH2 AND CH3 DOMAINS
- CLONE INTO DraIII-EcoRI SITES OF EXPRESSION VECTOR (VDJ-IgG4)

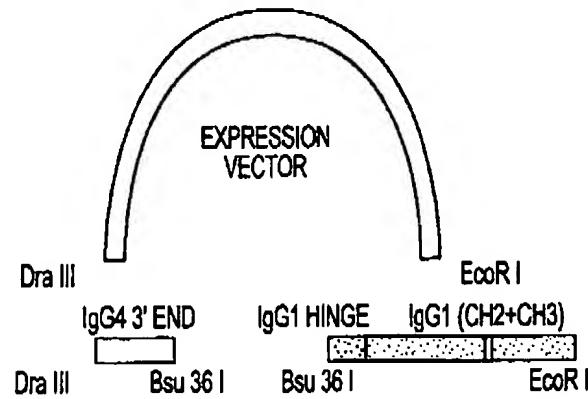


FIG. 2

Applicants: Michael Gallo et al. ABGX-2 CIP CPA
Application No.: 09/375,924
Filed: August 17, 1999
For: GENERATION OF MODIFIED MOLECULES
WITH INCREASED SERUM HALF-LIVES
Sheet 3 of 3

*Competition for binding with
protein AHRP*

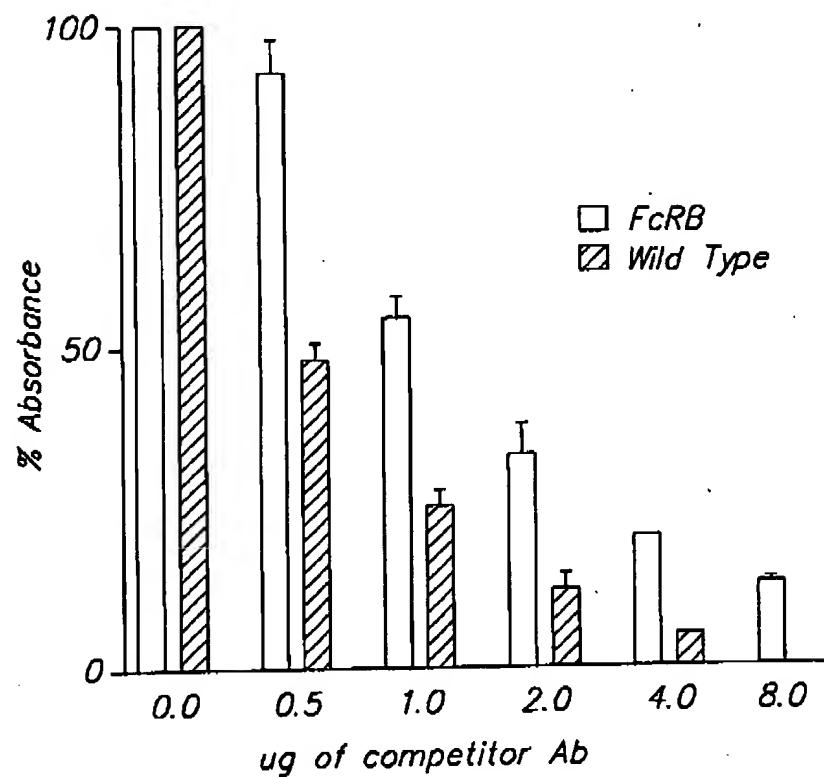


FIG. 3